

EDITORIAL COMMENT

Developing Medicines That Mimic the Natural Successes of the Human Genome



Lessons From *NPC1L1*, *HMGCR*, *PCSK9*, *APOC3*, and *CETP**

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Coronary heart disease (CHD) remains the leading cause of death in the industrialized world and will soon become so worldwide (1). Much research has focused on biological risk factors and developing medicines to modify them. However, remarkably few medicines (e.g., aspirin, statins, and antihypertensive agents) are proven to reduce CHD risk.

In several recent, large-scale, randomized-controlled trials, newly-developed medicines (e.g., dalcetrapib and darapladib) showed no benefit over placebo in reducing risk for coronary events (2-4). Often, these late-stage failures come after decades of research effort. Why were these results not anticipated? Two reasons stand out (5). First, the field has traditionally depended on in vitro or animal models; however, pre-clinical disease models often have limited ability to predict benefit in patients. For example, atherosclerosis in humans is the result of genetic makeup and decades of atherogenic stimuli; it is not surprising that this is difficult to recapitulate in cells or in nonhuman model organisms. Second, drugs that block a specific gene target are sometimes taken for many years; we often do not know the effect over such an extended period.

How can we more accurately anticipate whether a medicine will reduce risk for clinical CHD without adverse effects? In this issue of the *Journal*, Ference et al. (6) show that the human genome may be a valuable tool for prioritizing molecular targets in drug development. Medicines are typically designed to target a specific gene and its protein product. There is naturally occurring variation in nearly every gene, some of which resides in a specific drug's target gene. If this deoxyribonucleic acid (DNA) sequence variation modulates the gene's function or expression, then the phenotypic consequences of this variation in the human population could anticipate if a drug will safely reduce disease risk.

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This approach has now been applied to several drug-gene pairs. Some of the drugs are already in clinical use (ezetimibe targeting *NPC1L1*, statins targeting *HMGCR*) and some are in development (drugs targeting *PCSK9*, *APOC3*, and *CETP*). We will review each of these examples to understand the strengths and limitations of using genotype-phenotype correlations to anticipate a medicine's potential efficacy and safety.

Ezetimibe lowers plasma levels of low-density lipoprotein cholesterol (LDL-C) by blocking the Niemann-Pick C1-like 1 (*NPC1L1*) protein, a transporter allowing dietary cholesterol to enter the body from the intestinal lumen (7). Experiments in cells and mice confirmed that ezetimibe tightly binds to and blocks *NPC1L1*, and targeted deletion of *Npc1l1* in mice lowered plasma cholesterol and reduced atherosclerosis (8,9). In human volunteers, ezetimibe treatment blocked dietary cholesterol absorption by about 50% (10). However, addition of ezetimibe to background statin therapy failed to prevent atherosclerotic

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progression, as assessed by carotid intima-media thickness (11). These results raised uncertainty as to whether lowering LDL-C with ezetimibe would reduce risk for clinical CHD.

Ference et al. (6) mined the human genome to address this uncertainty. If ezetimibe successfully reduces risk for clinical CHD, DNA sequence variants that reduce *NPC1L1* function (i.e., mimicking ezetimibe action) would be expected to lower LDL-C and protect against CHD risk. The results of several recently-published, large, genome-wide searches for DNA sequence variants affecting plasma LDL-C (12,13) were leveraged by Ference et al. (6) to pinpoint 5 independent variants at or near the *NPC1L1* gene and develop a genetic score using these variants. Those with an *NPC1L1* gene score below the median had a 2.4 mg/dl lower LDL-C level than those with scores above the median. After testing the *NPC1L1* gene score in 108,376 individuals, the investigators found that those with the lower gene scores also had reduced risk for CHD (4.8% lower).

Late last year, we published findings (14) consistent with those of Ference et al. (6). Through sequencing of the protein-coding regions of *NPC1L1*, we found that approximately 1 in 650 persons carried a large-effect mutation (nonsense, splice-site, or frameshift; “null” mutations) that completely inactivated 1 of the 2 copies of *NPC1L1* present in each genome. Ezetimibe mimics heterozygous null mutations, as both reduce protein function by about one-half. We found that *NPC1L1* null mutation carriers had a 12 mg/dl lower LDL-C level (an effect size similar to ezetimibe treatment) and 53% lower risk for CHD. Together, both studies provide a strong therapeutic hypothesis that ezetimibe, a small-molecular inhibitor of *NPC1L1*, will not only lower LDL-C, but will also reduce risk for CHD.

The IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial; NCT00202878) has validated this hypothesis. To establish the clinical benefit and safety of ezetimibe, 18,144 high-risk individuals presenting with an acute coronary syndrome were randomized to simvastatin monotherapy versus simvastatin/ezetimibe combined therapy (15). The addition of ezetimibe significantly reduced the primary endpoint (cardiovascular death, myocardial infarction, unstable angina requiring hospitalization, coronary revascularization, or stroke), with a relative risk reduction of 6.4% and an absolute risk reduction of 2% over 7 years of treatment. This was the first trial to demonstrate that adding a nonstatin agent to a statin provides incremental clinical benefit. The average achieved LDL-C was 54 mg/dl with

ezetimibe/simvastatin dual therapy versus 70 mg/dl with simvastatin monotherapy. These data suggest that, in the setting of secondary prevention, we should target LDL-C to levels even lower than conventional thresholds.

Beyond ezetimibe-*NPC1L1*, there are now at least 4 other examples of drug-gene pairs affecting cardiovascular therapeutics, suggesting that this approach may be generalizable (Table 1 summarizes all 5 examples). Statins and their therapeutic target gene, 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), are another notable example. Numerous individual trials and comprehensive meta-analyses show that statins reduce the risk for either a first or recurrent CHD event (16). Although available for more than 2 decades, we only recently appreciated that statins lead to 2 important adverse events—modest weight gain and a small increase in the relative risk for type 2 diabetes (17,18). Could these adverse events have been anticipated using modern human genetic approaches?

We identified a naturally-occurring *HMGCR* gene variation associated with lower LDL-C that led to lower hepatic messenger ribonucleic acid expression and, thereby, a loss of gene function (19). Swerdlow et al. (18) demonstrated that those harboring an LDL-lowering allele at either of 2 *HMGCR* polymorphisms displayed higher body weight, increased fasting insulin, higher plasma glucose, and increased risk for type 2 diabetes. Clinical trial and genetic data are, thus, remarkably concordant in identifying both the efficacy and toxicity of statin therapy. Importantly, these data suggest that weight gain and glucose intolerance induced by statins is related to *HMGCR* inhibition, that is, an on-target class effect, rather than the characteristics of a single agent or off-target toxicity.

What might be the net effect on CHD of an intervention that lowers LDL-C while simultaneously increasing the risk of type 2 diabetes? From a meta-analysis of statin trials in the secondary prevention setting, it is clear that CHD risk is reduced with statin treatment (20). Importantly, human genetics suggests the same. In the study by Ference et al. (6), a genetic score of *HMGCR* polymorphisms was associated not only with lower LDL-C, but also with lower risk of CHD.

There are 3 examples of variation in target genes with therapies in development: *PCSK9*, *APOC3*, and *CETP*. About 1 in 50 black people carry a null mutation at the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, causing lifelong inactivation of 1 copy of the gene (21). These individuals have 28% lower LDL-C and are protected from CHD (88% lower

TABLE 1 Phenotypic Consequences of DNA Sequence Variation in Genes Targeted by Therapeutic Drugs									
Drug(s)	Gene Targeted by Drug(s)	Effect of Drug on Target Gene Function	DNA Sequence Variant(s) in Target Gene	Frequency of DNA Sequence Variant(s)	Anticipated Efficacy: Effect of DNA Variant on			Anticipated Adverse Events: Effect of DNA Variant on	
					Gene Function	Plasma Biomarkers	CHD Risk	Pleiotropic Phenotypes	
Ezetimibe	<i>NPC1L1</i>	Reduce	Gene score of 5 variants	Those above median in gene score	Reduce	↓ 2.3 mg/dl LDL-C	↓ 4.4%	None identified yet	
			Null mutations	1 in 650 cumulative carrier frequency	Reduce	↓ 12 mg/dl LDL-C	↓ 47%	None identified yet	
Statin	<i>HMGCR</i>	Reduce	Gene score of 3 variants	Those above median in gene score	Reduce	↓ 3.0 mg/dl LDL-C	↓ 5.2%		
			rs12916-T	60% frequency	Reduce mRNA expression	↓ 3.0 mg/dl LDL-C	↓ 4%	↑ 0.3 kg/m ² body mass index	
			rs17238484-G	77% frequency	?	↓ 2.3 mg/dl LDL-C	?	↑ 6% risk for T2D	
								↑ 0.3 kg/m ² body mass index	
								↑ 2% risk for T2D	
Alirocumab	<i>PCSK9</i>	Reduce	Y142X and C679X	2.6% carrier frequency among blacks	Reduce	↓ 28% LDL-C	↓ 88%	None identified yet	
Evolocumab									
Antisense oligonucleotide	<i>APOC3</i>	Reduce	R19X	1 in 150 cumulative carrier frequency	Reduce	↓ 39% TG	↓ 40%	None identified yet	
			IVS2+1G → A IVS3+1G → T A43T			↑ 22% HDL-C			
						↓ 16% LDL-C			
Anacetrapib	<i>CETP</i>	Reduce	D442G	6% carrier frequency in East Asians	Reduce	↑ 7 mg/dl HDL-C	Unknown	↑ 70% risk for AMD	
Evacetrapib		Reduce	rs3764261-A	32% minor allele frequency in whites	Reduce	↑ 3.4 mg/dl HDL-C	↓ 4%	↑ 19% risk for AMD	

AMD = age-related macular degeneration; CHD = coronary heart disease; DNA = deoxyribonucleic acid; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; mRNA = messenger ribonucleic acid; TG = triglycerides; T2D = type 2 diabetes.

risk), suggesting that therapies directed against *PCSK9* should not only lower plasma LDL-C, but should also reduce risk for CHD. Monoclonal antibodies directed against *PCSK9* reproducibly lower plasma LDL-C in humans, and several randomized trials are testing whether they reduce CHD in the secondary prevention setting (22). About 1 in 150 whites and blacks carry a null mutation, at the apolipoprotein C-III (*APOC3*) gene, leading to lifelong inactivation of 1 copy of the gene (23,24). These individuals display a favorable plasma lipid phenotype (lower triglycerides, lower LDL-C, lower remnant cholesterol, and higher high-density lipoprotein cholesterol [HDL-C]) and are protected from CHD (40% lower risk). These genetic data bolster enthusiasm for triglyceride-rich lipoproteins as a modifiable risk factor and suggest that therapies targeting *APOC3* should not only modify plasma lipids favorably, but should also lower CHD risk. Antisense oligonucleotide therapy targeting *APOC3* was shown to lower plasma triglyceride-rich lipoproteins in humans, and it remains to be seen whether it will lower CHD risk (25).

Finally, the cholesterol ester transfer protein (*CETP*) has been studied intensively as a therapeutic target. Several small molecules (anacetrapib, evacetrapib) inhibit *CETP*, leading to increased plasma HDL-C and decreased plasma concentrations of LDL-C, triglycerides, and lipoprotein(a) (26,27). About 6 in 100 individuals of East Asian ancestry carry a missense mutation at *CETP* (D442G), leading to loss of protein function and elevated plasma HDL-C levels (about 7 mg/dl higher) (28). It is still uncertain if *CETP* D442G carriers are protected from CHD. However, a recent study showed that they are at 70% increased risk for age-related macular degeneration (AMD), a leading cause of blindness in the United States (29). A common noncoding variant at *CETP* (rs3764261, 32% frequency) was previously shown to increase both HDL-C and AMD risk (about 3.4 mg/dl increase in HDL-C and 19% increase in AMD risk) (12,30). These genetic data suggest the hypothesis that *CETP* inhibition may lead to an on-target adverse event of AMD.

Randomized controlled trials of *CETP* inhibition are ongoing with anacetrapib (REVEAL [Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification]; NCT01252953) and evacetrapib (ACCELERATE [A Study of Evacetrapib in High-Risk Vascular Disease]; NCT01687998). These trials should clarify whether the genetic prediction of an adverse event will hold true. Of note, the effect of *CETP* D442G (or any germline genetic variant) on a phenotype relates to lifelong modulation, whereas the effect of a medicine typically reflects modulation

starting in adulthood; as such, one may not augur the other.

These five examples show the promise of using human genetics to prioritize molecular targets. How can we best expand the use of this approach? One recent idea is a U.S. Precision Medicine Initiative proposed by President Obama (31). Planning is underway for the recruitment of a new national research cohort linking genomic information to clinical phenotypes. At least 3 key elements seem important for optimally designing such a cohort for making observations like those in Table 1. Many null mutations that decrease disease risk and provide a strong therapeutic hypothesis will be extremely rare (often <1 in 1,000 frequency); thus, a large sample size will be required to confidently relate genotype to phenotype. A research cohort of approximately 1 million Americans is envisioned. Mutations vary in frequency by ethnicity and may be ethnic-specific (e.g., PCSK9 null mutations in African Americans); including a broad range of ethnic groups should enhance the probability of success. Finally, discovery of unanticipated biological connections, such as between HDL-C and eye disease, requires a rich set of clinical phenotypes for each participant.

Electronic health records could provide this, and a plan is being considered to link each volunteer's genomic information and electronic health record. When combined with creative analytics of the sort performed by Ference et al. (6), the Precision Medicine Initiative could become a transformative resource for understanding human biology and catalyzing the development of novel therapeutics.

It is now possible to systematically identify DNA sequence variations in therapeutic target genes, understand their phenotypic consequences, and use this information to anticipate a medicine's safety and efficacy. We have an unprecedented opportunity to leverage the genome's natural successes by scouring it for mutations that protect against disease with minimal adverse effects and developing medicines that mimic them.

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